IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Bahramian et al.
Appl. No:	09/472/558
File Date:	December 27, 1999
Invention:	MUTING GENE ACTIVITY USING A TRANSGENIC NUCLEIC ACID
Art Unit:	1632
Examiner:	Paras, Peter Jr.
Docket No.:	2498/101, formerly 2281/102

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CERTIFICATE OF FACSIMILE TRANSMISSION

I hereby certify that this correspondence is being transmitted by facsimile to the United States Patent and Trademark Office, Alexandria, VA, 22313, to Examiner Peter Paras, Art Unit 1632, fax number (703) 308-4242, telephone number (703) 308-8340, on August 6, 2003.

Barbara J. Carter, Ph.D.

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Honorable Commissioner of Patents
Alexandria, VA

DECLARATION OF MOHAMMAD B. BAHRAMIAN, PH.D. IN SUPPORT OF APPLICANTS' RESPONSE
[37 C.F.R. § 1.132]

Dear Sir:

In response to the Office Action mailed May 6, 2003, in the above-reference matter, I hereby declare as follows:

1. My name is Mohammad B. Bahramian, Ph.D. I am one of the inventors of the subject matter of the above patent application and the primary author on the 1999 Molecular and Cellular Biology publication (Bahramian et al., *Mol. Cell. Biol.* 19, 274-283, 1999) that was the first to show gene muting in a mammalian system. I am an author or co-author of a substantial number of publications involving (among other things) a PCR-based method for direct quantification of gene copy number in the nuclei of cells, ribitol dehydrogenase messenger RNA, synthesis and secretion of the human

growth hormone in *E. coli*, and double-stranded nucleic acid mediated gene silencing in mammals, and has a strong background in nucleic acid structure-function and gene expression. My further credentials are set forth in my Curriculum Vitae, which is attached as Exhibit A hereto.

2. I have read the action of May 6, 2003. This declaration is provided to clarify the record that someone of ordinary skill in the art would be able to practice the claimed invention based on the application as written (enablement).

THE INVENTION IS ENABLED BY THE SPECIFICATION

***General Information* - Post-transcriptional gene silencing (PTGS) by dsRNA**

3. The silencing of transgenes and of endogenous genes homologous to transgenes is a frequently observed phenomenon, known as co-suppression in plants and quelling in fungi (Meyer and Heidmann, 1994; Jorgensen, 1995; Matzke and Matzke, 1995; De Carvalho Niebel et al., 1995; Baulcombe and English, 1996; Cogoni and Macino, 1997), which occur by transcriptional and posttranscriptional processes. Transgene silencing and the related effects had also been described in invertebrate animals (Pal Bhadra et al., 1997; Chaboissier et al., 1998; Fire et al., 1998; Kennerdell and Carthew, 1998; Ruiz et al., 1998). Posttranscriptional gene silencing (PTGS) is a general term that applies to RNA interference (RNAi) in animals, and to some types of virally- and transgene-induced silencing in plants. The transcription of the gene is unaffected; however, gene expression is lost because mRNA molecules become unstable (Hammond et al., 2001).

4. The term RNA interference, or "RNAi", was initially coined by Fire and coworkers (Fire et al., 1998) to describe the observation that long double-stranded RNA (dsRNA) can block gene expression when it is introduced into worms (Zamore et al.,

2000). RNAi by long dsRNA was subsequently demonstrated in other small animals, including flies (Kennerdall and Carthew, 1998) and trypanosomes (Ngo et al., 1998) and was linked to gene silencing phenomena in plants, co-suppression, (Vaucheret et al., 1998; Waterhouse et al., 1998) and the fungus *Neurospora*, quelling, (Cogoni et al, 1997). Curiously, neither co-suppression nor RNAi could be demonstrated in higher animals, particularly in mammalian cells. But it was known that dsRNA >30 bp in the cytoplasm of mammalian cells can trigger profound physiological reactions that lead to the induction of interferon synthesis (Stark et al., 1998).

5. The first example of transgene-induced gene silencing was provided in normal and transformed rodent cell lines, which occurred by both transcriptional and posttranscriptional mechanisms (Bahramian and Zarbl, 1999). That report, while uncoupling transcriptional and posttranscriptional mechanisms of transgene-induced muting, implicitly, by default, linked the potent and specific mRNA degradation in mammalian cells, resulting from PTGS, to the *in vivo* production of short dsRNA that act as the intermediary molecule of communication between the silenced genes in the cell nucleus and the cognate mRNA in the cytoplasm.

6. Following that discovery, the mediators of sequence-specific mRNA degradation were found to be 21- and 22-nucleotide small interfering RNAs (siRNAs) generated by ribonuclease III cleavage from longer dsRNAs (Hammond et al., 2000; Zamore et al., 2000; Bernstein et al., 2001; Elbashir et al., 2001). Thus, it was discovered what separates RNAi in higher animals from lower animals and plants was the size of effective dsRNA. Furthermore, Bahramian & Zarbl's work (1999) showed that the reason for inability to show plant- and lower animal-type co-suppression in mammalian cells is

certain distinctions between the silencing mechanisms in plant and lower animals versus higher animals, even though they are related by RNAi (discussed below).

Distinction of muting from co-suppression

7. Co-suppression and the related mechanisms had been observed in plants, *Drosophila*, fungi and *C. elegans*, but NEVER occurred in higher animals, particularly in mammals. Characteristically, following STABLE integration of one or more copies of homologous transgene(s), reduction in expression of the endogenous gene and the transgenes occur in reciprocal fashion (thus, co-suppression). This trans-suppression requires transcription of the transgenes but is independent of the specific promoter sequence used to drive the transcription.

8. By contrast, if one stably transfects any transgene that is homologous to an endogenous gene into any higher animal, such as a mammal, one would never observe co-suppression. For example, integration of the full-length pro-α1(I) collagen gene construct (pWTC1) into rodent cell genome results in the transgene expression levels indistinguishable from that of the endogenous gene; therefore, no 'co-suppression' occurs.

9. In addition to the fact that integration of the transgene(s) into a mammalian genome does not result in co-suppression, there are other significant differences between co-suppression and muting. Co-suppression requires the transcription of the transgenes and is influenced by the level of this expression. However, in our report, at least one construct, the full-length procollagen (pWTC1) produced no detectable mRNA during transient transfection, yet was capable of reducing dramatically the expression of the homologous endogene. Progressive 5' promoter deletion constructs attached to a single

reporter gene at a single location express the reporter gene to different extents, due to representation of various enhancers and repressors. Yet, each of those constructs suppresses the transcription of the endogenous gene to exactly the same level, i.e., level of endogenous gene muting is unrelated to the strength of the transgene promoter. In spite of the differences, co-suppression and gene muting are linked somehow through PTGS.

10. In summary, the knowledge generally available to those skilled in the art at the time this application was such that the claims of the application, as written, would be understood are fully enabled. One skilled in the art could readily follow the suggestions for how to identify muting nucleic compositions for other genes in other systems and practice the claimed invention without undue experimentation. Please consider these comments in conjunction with the response submitted herewith.

11. I hereby declare that all statements made herein are of my own knowledge and that all statements made on information and belief are true; and further that these statements are being made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

M.B. Bahramian

Mohammad B. Bahramian, Ph.D.

Dated: August 6, 2003

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EXHIBIT A
SHORT BIO OF DR. B. BAHRAMIAN

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SUMMARY:

B.Sc. (Alberta), M.Sc. (McGill), Ph.D. (Imperial College of Science & Technology, London, UK), Sanofi Visiting Scientist and NIH Fellow (Massachusetts Institute of Technology), Head of Laboratory (Pasteur Institute, France), Director of Molecular Biology (Sanofi-Elf Bio-Recherches, France). Bahramian is the co-inventor of 'Muting Gene Activity Using a Transgenic Nucleic Acid, which comprises, but not limited to, "RNAi" technologies. He has had a varied and productive career, comprising ten years of research and development in Biotechnology/Pharmaceuticals. He is the author of over 30 scientific publications and patent applications in the biological sciences, and has a strong background in nucleic acid structure-function and gene expression.

SAMPLING OF SCIENTIFIC ACHIEVEMENTS:

- Pioneered epigenetic double-stranded nucleic acid mediated gene silencing in mammals.
- Engineered coordinated synthesis and secretion of the human growth hormone in *E. coli*, the first authentic eukaryotic protein produced in bacteria in this way, leading to patents.
- Isolated ribitol dehydrogenase messenger RNA of *Klebsiella aerogenes*, the first fully intact bacterial mRNA purified for any cytoplasmic or metabolic protein.
- Invented a PCR-based method for direct quantification of gene copy number in the nuclei of cells, which enables comparative gene expression studies between different cell lines.

EXPERIENCE:

INDEPENDENT SCIENTIST: 2001 - 2003.
Developing patents, business plan, and commercialization of an intellectual property concerned with a fundamental mechanism of control of gene expression that is enabling for selective inhibition of gene activity in mammalian cells. The invention is useful in developing gene-based therapies and 'functional genomic' studies. It uses therapeutic DNA, RNA and the respective analogs.

VION PHARMACEUTICALS, INC., New Haven, CT: 1998 - 2000.
Senior Research Scientist Development: Responsible for technology transfer and establishing QC/bio-analytical facility in compliance with the FDA-approved cGLP/cGMP guidelines for VION'S platform technology, a bacterial gene delivery system for anti-cancer therapies. Accomplished all of the objectives including:

Transferring technology from Research to Development and facilitating communications.
Evaluating, developing and validating methods for testing the identity, purity, safety and efficacy of the drug product.
Managing stability programs of the drug product lots.
Preparing FDA related documents, including the CMC sections of INDs, study protocols, validation reports, test procedures, and SOPs.
QC testing, evaluating and interpreting preclinical and clinical data.
Collaborating with Manufacturing, to define conditions for the drug administration.

INDEPENDENT BIO-TECHNOLOGICAL CONSULTING: 1996 - 1997.

BRIEF SUMMARY OF 1999 MOL. CELL. BIOL. ARTICLE, AND ITS AUTHORS

Muting Gene Activity Using A Transgenic Nucleic Acid

Inventors: M. Bahman Bahramian, Ph.D.* and Helmut Zarbl, Ph.D.†

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Targeted inhibition of gene expression has been a long-felt need in biotechnology and in development of gene-based therapies. In the past decade, several companies have tried to tap the potential of RNA-based interference mechanisms. The latest and the most promising entry into the field is RNA interference (RNAi), which uses the introduction of short, double-stranded RNA into a cell to inhibit gene expression in a sequence-dependent fashion. Nevertheless, RNAi mechanisms are still not fully elucidated, compound stability and delivery are unsolved, and the side effects are unknown. Thus, while RNAi already has been adopted as a functional genomics tool, its utility as a therapeutic platform remains unknown.

'Muting gene activity using a transgenic nucleic acid' (gene muting) is the title of an invention and an unpublished US patent application, which comprises RNAi and has priority date over all RNAi patents pertaining to mammalian cells. Gene Muting was identified as a natural process featuring 'coordinated cellular mechanisms for efficient inhibition of transcription and degradation of the transcripts of an endogenous gene in response to either particular physiological signals or ectopic introduction of nucleic acids homologous to certain regions of the target gene'. The invention selectively inhibits the expression of an endogenous gene or a transgene in mammalian cells by transient use of 'muting nucleic acid sequences' that are homologous to certain regions of the target gene.

Advantages of Gene Muting:

- Is independent of integration and recombination of the muting nucleic acid.
- With the choice of muting nucleic acid as DNA, RNA or analogs, muting can specifically inhibit mRNA transcription and/or degrade the target-gene mRNA post-transcriptionally. In contrast, RNAi technologies only act post-transcriptionally, without the ability to inhibit transcription. Thus, muting provides tighter control of gene expression.
- By using the appropriate regulatory DNA sequences as the muting nucleic acid, one could choose to inhibit transcription and induce degradation of the mRNA of the target gene in a highly specific and efficient manner without the need for transcription of the muting molecule. This is an enormous advantage since therapy is often challenged by double jeopardy of vector delivery and transgene transcription.
- The invention is unique in offering the choice of transcriptional and/or post-transcriptional gene silencing and comprising RNAi technologies.
- The invention is useful in transgenic animal research requiring knocking out the function of a gene, without the need for expression or recombination of the transgene.
- Muting can work independent of the level of target gene expression and the cell type.

- Muting offers advantages over RNAi and the related technologies, in efficacy, stability, cost, and the range of applications.

BIOGRAPHIES OF THE AUTHORS

Bahman Bahramian is an independent scientist/entrepreneur, working from Branford, Connecticut, on development of a business based on an IP that originated at Massachusetts Institute of Technology. At MIT, he worked with Professor Uttam L. RajBhandary (1987-88) as a Visiting Scientist from Sanofi-Elf BioRecherches, on structure-function relationship of initiator tRNA. Later (1991-95), he worked as a Postdoctoral Research Fellow with Professor Helmut Zarbl on mechanisms of v-fos-induced cellular transformation in fibroblast cells, where the 'Gene Muting Technology' [IP] was discovered. Bahramian has enjoyed a diverse career in Molecular Biology/ Biochemistry and Gene Expression. For example, during his doctoral research with Professor Brian S. Hartley, FRS, at Imperial College London, on 'experimental enzyme evolution project', he isolated and characterized ribitol dehydrogenase mRNA of *Klebsiella aerogenes*, the first fully intact bacterial mRNA isolated for any metabolic or cytoplasmic protein. At Pasteur Institute, though not generally known, he engineered coordinated synthesis and secretion of the human growth hormone in *E. coli*, the first authentic eukaryotic protein produced in bacteria in this way, leading to patents. In collaboration with the University of Ottawa scientists, he discovered a single nucleotide mutation that determines the 'milling property' of flour, whether it should go into pastry or bread. Bahramian graduated from Imperial College of Science & Technology with a Ph.D. in Biochemistry and Molecular Biology. He has a Master of Science from McGill

University in Genetics and a B.Sc. from University of Alberta. He has over thirty years of academic and industrial research and development experience.

Helmut Zarbl is a Full Member of Fred Hutchinson Cancer Research Center. He is also an Affiliate Professor at University of Washington, School of Medicine and School of Public Health and Community Medicine. Zarbl is Principle Investigator FHCRC/UW Toxicogenomics Consortium National Institute of Environmental Health Sciences. He is Director, Environmental Carcinogenesis Core, University of Washington School of Public Health and Community Medicine, Center for Ecogenetics and Environmental Health. Zarbl is a graduate of McGill University with a Ph.D. in Biochemistry. Prior to joining FHCRC, he was Assistant and Associate Professor (1987-1994), Massachusetts Institute of Technology, School of Science & Whittaker College, Toxicology.